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CASE REPORT

FISH studies in a girl with sporadic aniridia and an apparently balanced *de novo* t(11;13)(p13;q33) translocation detect a microdeletion involving the WAGR region

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Abstract

Conventional cytogenetic studies on a female infant with sporadic aniridia revealed what appeared to be a balanced *de novo* t(11;13)(p13;q33) translocation. Fluorescence *in* situ hybridization (FISH) investigations, however, detected the presence of a cryptic 11p13p14 deletion which included the WAGR region and involved approximately 7.5 Mb of DNA, including the PAX6 and WT1 genes. These results account for the patient's aniridia, and place her at high risk for developing Wilms' tumour. The absence of mental retardation in the patient suggests that the position of the distal breakpoint may also help to refine the mental retardation locus in the WAGR contiguous gene syndrome (Wilms', aniridia, genital anomalies and mental retardation).

INTRODUCTION

Aniridia is a rare (1:50,000) congenital condition that results in an absence of the iris. Aniridia leads to visual impairment and blindness in severe cases. The location of the human aniridia gene was first suggested by the observation of an association of interstitial 11p13 deletions with aniridia and Wilms' tumour (Riccardi *et al.*, 1978; Francke *et al.*, 1979). Isolated aniridia cases were found retrospectively to be associated with other 11p13 aberrations (Tommerup, 1993). Furthermore, refined cytogenetic and molecular studies have mapped and identified both the human aniridia gene (PAX6) and a Wilms' tumour predisposition locus (WT1) to distal 11p13 (Call *et al.*, 1990; Gessler *et al.*, 1990; Ton *et al.*, 1991).

Conventional and molecular cytogenetic studies have shown that approximately 14% of isolated aniridia (sporadic and familial) result from deletions in PAX6 which may extend proximally to include WT1 (Crolla *et al.*, 1997). DNA studies have shown that the majority of sporadic aniridia is caused by intragenic mutations of PAX6 resulting in haploinsufficiency of the PAX6 gene product (Jordan *et al.*, 1992; Glaser *et al.*, 1992; Hanson *et al.*, 1993; Axton *et al.*, 1997). In very rare cases of balanced structural chromosomal rearrangements with 11p13 breakpoints, the mutant phenotype has probably resulted from a position effect following disruption of the chromatin domain distal to the PAX6 locus. This disruption impairs normal gene function and by an unknown mechanism results in functional haploinsufficiency (Fantes *et al.*, 1995a; Crolla *et al.*, 1996).

The diagnosis of sporadic aniridia in early infancy strongly indicates an immediate search for an 11p13 deletion. Should the deletion include the WT1 locus, there may be a high risk of developing Wilms' or, much more less frequently, gonadal tumours. Even if a deletion is detected, it is difficult to predict the effect of the deletions on developmental outcome. Developmental delay and mental retardation form one of the main clinical components of the WAGR contiguous gene syndrome (Wilms, aniridia, genital anomalies and mental retardation).

We report the case of a young girl with sporadic bilateral aniridia, who, was shown by conventional cytogenetic analysis to be carrying a *de novo* balanced translocation t(11;13)(p13;q33). Fluorescence *in situ* hybridization (FISH) investigations using whole chromosome paints, cosmids and YACS from chromosome 11 and 13 revealed a cryptic deletion involving approximately 7.5 Mb of DNA incompassing the WAGR region.

CASE REPORT

The daughter of a non-consanguineous young couple (TAA; IFF No.054610) was born at term after an uncomplicated pregnancy. Her weight and length at birth were 2,520 g and 45 cm, respectively. At four months of age bilateral glaucoma was diagnosed and bilateral aniridia was found at surgery. She stood and walked with support at 9 months of age, craddled and sat by herself at 11 months, and walked unaided at 16 months. At this age, no evidence of intellectual impairment was noted, an observation confirmed by a paediatric clinical psychologist. Her slight deviation from normal standard tests applied (BSID-II) was

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attributed to her visual impairment. At 2 years and 6 months her height and weight were normal, and she was not dysmorphic (Figure 1). Regular abdominal ultrasound scans were performed and no abnormality was discovered.

CYTOGENETIC AND FISH INVESTIGATIONS

Cytogenetic investigations were performed with PHA-stimulated lymphocyte cultures following standard procedures. FISH protocols were performed using lymphocyte and lymphoblastoid cell culture metaphases and summarised as follows: the 11p cosmids (telomeric -> centromeric, i.e., CO8160, F1238, FO2121, FAT5 (PAX6), B2.1 (WT1), c1-11-458 and c1-11-474 - (see Fantes *et al.* 1995b, for detailed mapping information)), and the 11p sub-telomeric YAC, HTY3219 (kindly provided by Helen Donis-Keller), were digoxigenin labelled using a nick-translation kit following the manufacturer's protocol (Boehringer Mannheim). In addition, a directly labelled (fluorogreen, Amersham) alphoid centromere 11 specific repeat probe (D11Z1) was used to identify the normal and der(11)s, respectively. Thirty to fifty nanograms of labelled cosmid/YAC DNA and 50 ng of directly labelled D11Z1 was mixed with 3 µg of Cot-1 DNA (Gibco BRL), resuspended in hybridisation mixture comprising 2X SSC, 50% w/v deionised formamide and 10% w/v dextran sulphate. Probe and competitor DNA were denaturated for 10 min at 72°C and re-annealed at 37°C for 20-30 min prior to being added to the denatured chromosomal DNA. Hybridizations were carried out for a minimum of 16-18 h at 37°C and the hybridised slides were washed using under stringent conditions using 2X SSC at room temperature for 5 min, two 5-min washes in 50% formamide/50% 2X SSC at 42°C, and a final 2X SSC wash at 42°C for 5 min. The whole chromosome flow-sorted 11 and 13 paint libraries (Cambio) were used according to the manufacturer's instructions. Digoxigenin-labelled probes were detected using one layer of anti-digoxigenin-Rhodamine (TRITC). The chromosomal DNA was counterstained with 0.05 mg/ml DAPI suspended in an anti-fade solution (Vectashield, Vector Labs, UK) and examined using a Carl Zeiss Axioskop epifluorescent microscope fitted with Chroma Technology's Pinkel fluorescent No. 83 filter series. Images were captured using a cooled CCD camera and the digitised data were visualised and analysed using Smartcapture software (Vysis UK). Cosmid/YAC signals were seen as red and the alphoid repeat signal as green. A minimum of 5 metaphases were scored after hybridisation with each probe or probe combination. Details of the physical location of the 11p cosmids used and their relative distances have been published in Fantes *et al.* (1995b).

RESULTS

Conventional cytogenetic investigations using lymphocyte cultures revealed an abnormal karyotype [46,XX,t(11;13)(p13;q33)] in all 100 cells analysed (Figure 2). The

Figure 1 - Proband at 2 years of age. Note left aniridia and right corneal opacity without facial dysmorphies.

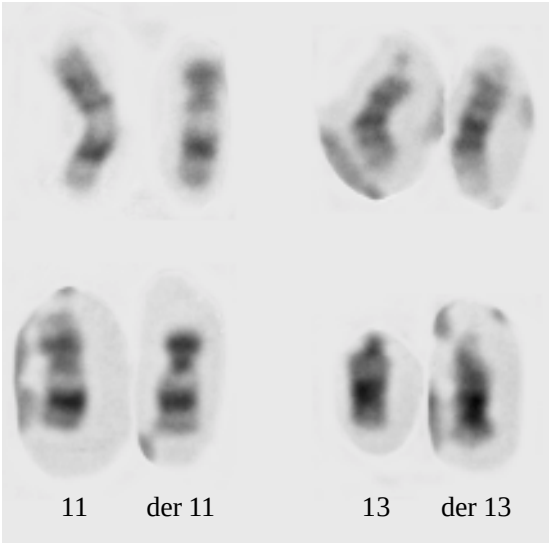

Figure 2 - Proband's G-banded partial metaphases showing balanced 46,XX,t(11;13)(p13;q33) *de novo* translocation.

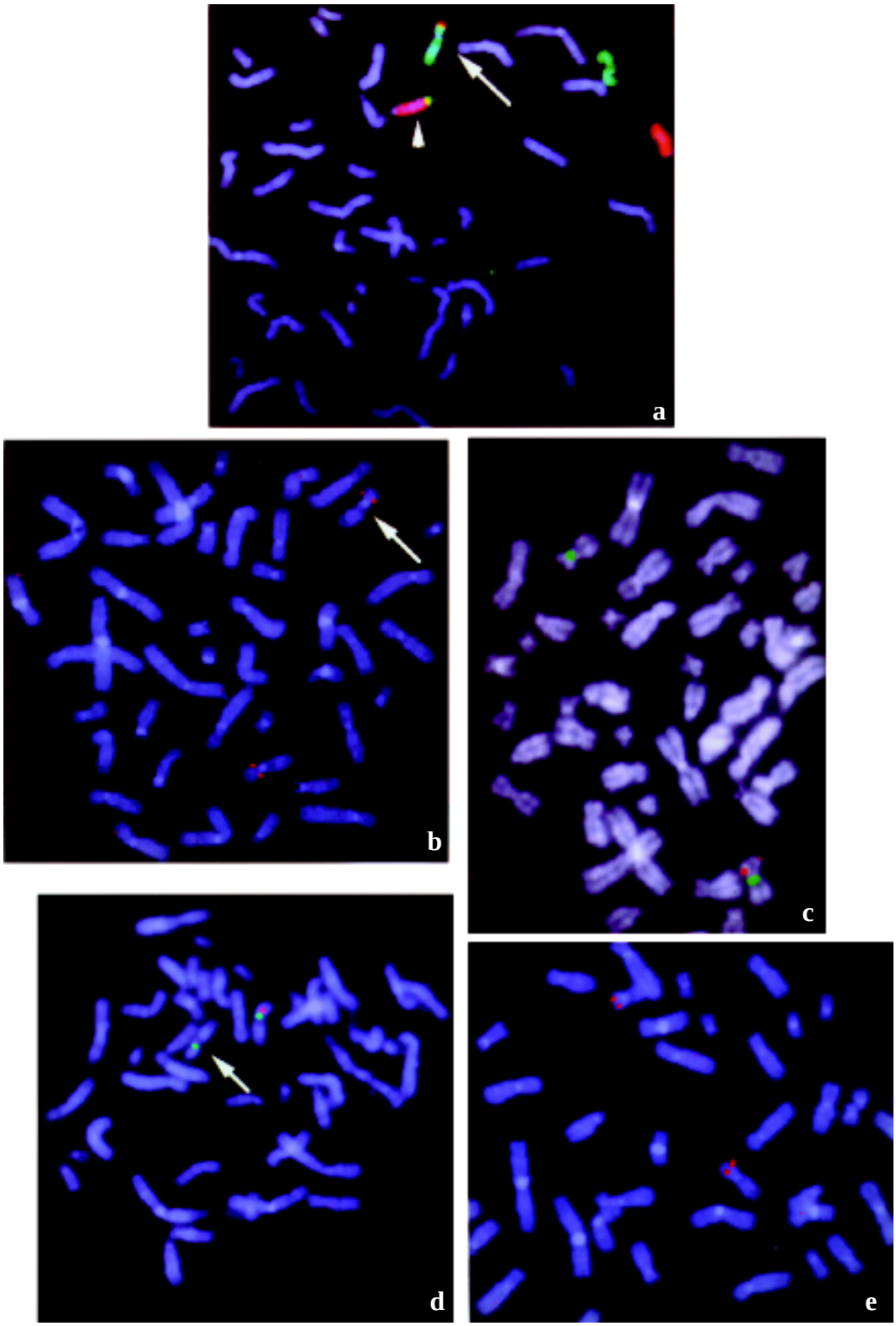

Figure 3 - Examples of the results obtained following FISH with dual colour whole chromosome 11 and 13 paints (a) and locus specific cosmids (b,c,d,e): (**a**) Dual colour painting using a directly FITC-labelled (green) chromosome 11 paint and a biotin-labelled rhodamine-stained (red) chromosome 13 paint. Both the der(11) - arrow - and the der(13) - arrowhead - show clear evidence of a reciprocal exchange of chromatin from distal 11p and distal 13q, respectively. Furthermore, the painting result suggests that the chromatin missing from the interstitial segment of 11p has not been involved in an inter-chromosomal rearrangement (see text). (**b**) Cosmid 1-11-474 which maps to 11p12. Both the normal and der(11) - arrow - show signal with this probe. (**c**,**d**) Cosmids B2.1 and FAT5 which maps to WT1 and PAX6, respectively. The cosmid signal is red (rhodamine) and the 11 centromeres are identified by the alphoid repeat probe D11Z1 (labelled green with FITC). The der(11) - arrow in d - are deleted for WT1 and PAX6. (**e**) Cosmid F1238 which maps to 11p14.2. Both the normal and der(11) show signal with this probe.

parental karyotypes were normal. FISH using the whole chromosome 11 and 13 paints confirmed a translocation between the short arm of chromosome 11 and the distal long arm of chromosome 13 (Figure 3a) and the 11p sub-telomeric repeat yHTY3219 was present on the normal 11 and also on the distal tip of the der(13) indicating that distal 11p is involved in the translocation. FISH studies with the cosmids from distal 11p13 showed that FO2121, FAT5 (PAX6 - Figure 3d), and B2.1 (WT1 - Figure 3c) were deleted in the der(11), indicating an interstitial deletion of at least 800 kb. Further FISH studies with probes cloned from regions flanking 11p13 have shown that the cryptic deletion extends approximately 7.5 Mb with a proximal breakpoint lying between c1-11-474 (p12 - Figure 3b) and c1-11-458 (proximal p13). The distal breakpoint occurred between C08160 (p13/p14 border) and F1238 (mp14.2 - Figure 3d). No evidence of hybridization of any of the tested cosmids to other chromosomal locations was identified, suggesting that the material deleted in the der(11) had not been translocated elsewhere. The proband's karyotype can therefore be characterised as follows (ISCN, 1995):

46,XX,t(11;13)(p13;q33)*de novo*.ish t(11;13)(yHTY 3219-,wcp13+,E06182+,F1238+,C08160-,FO2121-, FAT5-,B2.1-,D11S672-,c1-11-474+,D08153+,D11Z1+; wcp11+,yHTY3219+).

A fibroblast cell line is available under the code IFF No. 054610 CGM /96 (Rio de Janeiro, Brazil) and lymphoblastoid cell lines from the European Cell & Culture Collection, Division of Biologics, Porton Down, Salisbury, SP4 0J4, UK (Proband DD2737; Mother DD2741; Father DD2736).

DISCUSSION

In our patient, besides an apparently balanced reciprocal 11p;13q translocation, several 11p cosmids showed an associated deletion extending over approximately 7.5 Mb which included the ~800 kb of the WAGR contiguous gene region. The FISH results were compatible with a proximal breakpoint in the der(11) distal to the EXT2 locus and recently mapped to an approximately 20 cM region of 11p→p13 (Bartsch *et al.*, 1996). The distal breakpoint mapped between CO8160 and F1238 and was therefore distal to D11S302 (239FB) probes. Using Northern blot analysis, Schwartz *et al.* (1995) showed that this gene is prominently expressed in fetal brain cortex. Consequently, it has been proposed as a possible candidate gene for the mental retardation phenotype associated with WAGR. The deletion observed in our patient extends telomerically to F1238 (interval XV, Fantes *et al.*, 1995b) and may have also included the BDNF (brain-derived neurotrophic locus), which has also been implicated in the mental retardation phenotype (Hanson *et al.*, 1993; Fantes *et al.*, 1995b). Our patient, however, was not mentally retarded, so detailed mapping of the distal breakpoint may be significant in defining the location of other possible retardation associated gene(s).

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RESUMO

O estudo citogenético convencional em uma menina com aniridia esporádica resultou em uma aparente translocação balanceada t(11;13)(p13;q33) *de novo*. Entretanto, o estudo citogenético pela hibridação *in situ* fluorescente (FISH) detectou a presença de uma deleção críptica 11p13p14, incluindo a região WAGR e envolvendo aproximadamente 7.5 Mb de DNA, deletando os genes PAX6 e WT1. Estes resultados correlacionam-se com o quadro clínico da paciente e a coloca em alto risco de desenvolver tumor de Wilms. A ausência de retardo mental na paciente indica que a posição distal do ponto de quebra poderá refinar o mapeamento do locus retardo mental na síndrome de genes contíguos WAGR (Wilms, aniridia, anomalias genitais e retardo mental).

REFERENCES

Axton, R., Hanson, I., Danes, S., Sellar, G., van Heyningen, V. and **Prosser, J.** (1997). The incidence of PAX6 mutation in patients with simple aniridia: an evaluation of mutation detected in 12 cases. *J. Med. Genet. 34*: 279-286.

Bartsch, O., Wuyts, W., Van Hul, W., Hecht, J., Meinecke, P., Hogue, D., Werner, W., Zabel, B., Hinkel, G., Powell, C., Shaffer, L. and **Willems, P.** (1996). Delineation of a contiguous gene syndrome with multiple exostoses, enlarged parietal foramina, craniofacial dysostosis, and mental retardation, caused by deletions on the short arm of chromosome 11. *Am. J. Hum. Genet. 58*: 734-742.

Call, K.M., Glaser, T., Ito, C.Y., Buckler, A.J., Pelletier, J., Haber, D., Rose, E., Kral, A., Yeger, H., Lewis, W.H., Jones, C. and **Housman, D.E.** (1990). Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell 60*: 509-520.

Crolla, J.A., Cross, I., Atkey, N., Wright, M. and **Oley, C.A.** (1996). FISH studies in a patient with sporadic aniridia and t(7;11)(q31.2;p13). *J. Med. Genet. 33*: 66-68.

Crolla, J.A., Cawdery, J.E., Oley, C.A., Young, I.D., Gray, J., Fantes, J. and **van Heyningen, V.** (1997). A FISH approach to defining the extent and significance of deletions at the WAGR locus. *J. Med. Genet. 34*: 207-212.

Fantes, J., Redeker, B., Breen, M., Boyle, S., Brown, J., Fletcher, J., Jones, S., Bickmore, W., Fukushima, Y., Mannens, M., Danes, S., Van Heyningen, V. and **Hanson, I.** (1995a). Aniridia-associated cytogenetic rearrangements suggest that a position effect may cause the mutant phenotype. *Hum. Mol. Genet. 4*: 415-422.

Fantes, J.A., Oghene, K., Boyle, S., Danes, S., Fletcher, J.M., Bruford, E.,

Williamson, K., Seawright, A., Schedl, A., Hanson, I., Zehetner, G., Bhogal, R., Lehrach, H., Gregory, S., Williams, J., Little, P., Sellar, G., Hoovers, J., Mannens, M., Weissenbach, J., Junien, C., Van Heyningen, V. and **Bickmore, W.** (1995b). A high-resolution integrated physical, cytogenetic, and genetic map of human chromosome 11: Distal p13 to proximal p15.1. *Genomics 25*: 447-461.

Francke, U., Holmes, L.B., Atkins, L. and **Riccardi, V.M.** (1979). Aniridia-Wilms' tumor association: evidence for specific deletion of 11p13. *Cytogenet. Cell. Genet. 24*: 185-192.

Gessler, M., Poustka, A., Cavanee, W., Neve, R.L., Orkin, S.H. and **Bruns, G.A.P.** (1990). Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. *Nature 343*: 774-778.

Glaser, T., Walton, D.S. and **Maas, R.L.** (1992). Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. *Nat. Genet. 2*: 232-239.

Hanson, I.M., Seawright, A., Hardman, K., Hodgson, S., Zaletayev, D., Fekete, G. and **Van Heyningen, V.** (1993). PAX6 mutations in aniridia. *Hum. Mol. Genet. 2*: 915-920.

ISCN (1995). *An International System for Human Cytogenetic Nomenclature* (Mitelman, F., ed.). Karger, Basel.

Jordan, T., Hanson, I.M., Zaletayev, D., Hodgson, S., Prosser, J., Seawright, A., Hastie, N. and **Van Heyningen, V.** (1992). The human PAX6 gene is mutated in two patients with aniridia. *Nat. Genet. 1*: 328-332.

Riccardi, V.M., Sujansky, E., Smith, A.C. and **Francke, U.** (1978). Chromosomal imbalance in the aniridia-Wilms' tumor association: 11p interstitial deletion. *Pediatrics 61*: 604-610.

Schwartz, F., Neve, R., Eisenman, R., Gessler, M. and **Bruns, G.** (1994). A WAGR region gene between PAX-6 and FSHB expressed in fetal brain. *Hum. Genet. 94*: 658-664.

Schwartz, F., Eisenman, R., Knoll, J., Gessler, M. and **Bruns, G.** (1995). cDNA sequence, genomic organisation, and evolutionary conservation of a novel gene from the WAGR region. *Genomics 29*: 526-532.

Tommerup, N. (1993). Mendelian cytogenetics. Chromosome rearrangements asssociated with Mendelian disorders. *J. Med. Genet. 30*: 713-727.

Ton, C.C.T., Hirvonen, H., Miwa, H., Weil, M.M., Monaghan, P., Jordan, T., Van Heyningen, V., Hastie, N.D., Meijers-Heijboer, H., Drechsler, M., Royer-Pokora, B., Collins, F., Swaroop, A., Strong, L.C. and **Saunders, G.F.** (1991). Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. *Cell 67*: 1059-1074.

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